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European Journal of Medicinal Chemistry 39 (2004) 827–834
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Original article

Esters, amides and substituted derivatives of cinnamic acid: synthesis, antimicrobial activity and QSAR investigations

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Received 18 September 2003; received in revised form 3 June 2004; accepted 9 June 2004

Available online 11 September 2004

Abstract

A series of esters ($\mathbf{I_{a–k}}$), substituted derivatives ($\mathbf{II_{a–d}}$) and amides ($\mathbf{III_{a–q}}$) of cinnamic acid were synthesized and evaluated as antibacterial and antifungal agents. All the derivatives belonging to the series **I, II** and **III** showed antimicrobial activity comparable to the standard. Compounds $\mathbf{I_f}$ and $\mathbf{II_c}$ proved to be the most effective compounds. Quantitative structure–activity relationship (QSAR) investigation with multiple linear regression analysis was applied to find a correlation between different calculated physicochemical parameters of the compounds and biological activity. The quantitative models relating the structural features of cinnamic acid derivatives $\mathbf{I_{a–k}}$, $\mathbf{II_{a–d}}$ and $\mathbf{III_{a–q}}$ and their antimicrobial activity showed that Gram negative *Escherichia coli* and *Candida albicans* (fungus) were the most sensitive microorganisms.

Keywords: Cinnamic acid; Antibacterial activity; Antifungal activity; QSAR

1. Introduction

Pursuing the studies on cinnamic acid derivatives with antibacterial and antifungal activity [1–6], as a part of our research project on sorbic, cinnamic and ricinoleic acid derivatives with potential antimicrobial activity, we recently reported [7] the synthesis and the correlation between physicochemical properties and biological activity of compounds belonging to the series $\mathbf{I_{a–i}}$ (Table 1).

Since it is well known that cinnamic acid plays an important role for the antimicrobial activity [8–11], in the present paper the evaluation of the in vitro antimicrobial activity of compounds belonging to the series **I–III** (Table 1) and the investigation of the relationship between their physicochemical properties and microbiological effects have been discussed.

Several studies have pointed out the major role of quantitative structure–activity relationship (QSAR) in the development of antimicrobial agents [12–14] and in particular it has already been suggested [15] that QSAR study of mono-, di- and tri-substituted cinnamic acids against *Listeria monocytogenes* indicated the importance of lipophilicity parameter on activity. The mode of action [16,17] concerning antibacterial activity involves an unspecific interaction (strictly related to the hydrophobic character of the molecules) on protein thiol groups. Moreover, concerning antifungal activity, many compounds exert a membrane action, strictly related to lipophilicity [18,19]. QSAR study of benzene sulphonamide flouroquinolones [12] showed a linear correlation of antibacterial activity with electronic distribution along with steric parameters. From the above consideration, it was decided to find a correlation between lipophilicity parameters along with steric and electronic parameters of cinnamic acid derivatives and their antimicrobial activity. Following general antibacterial screening (series $\mathbf{I_{a–I}}$), indicating sufficiently encouraging effects [7], three series (**I, II, III**) were selected for an extensive evaluation of their antimicrobial effects against representative bacterial and fungal microorganisms.

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doi:10.1016/j.ejmech.2004.06.013

Table 1
Physicochemical properties of cinnamic acid derivatives

C_6H_5–CH=CH–COOR($\mathbf{I_a}$–$\mathbf{I_k}$)		C_6H_4(R)–CH=CH–COOH($\mathbf{II_{a–b}}$)		C_6H_5–CH(R)–CH(R)–COOH(**IIc–d**)			C_6H_5–CH=CH–CO–R($\mathbf{III_{a–q}}$)		
Compound code	R	Molecular formula	Molecular weight	m.p./b.p.	R_f value	Yield (%)	C(Calc.) (%)	Anal. H(Calc.) (%)	N (Calc.) (%)
I	H	$C_9H_8O_2$	148	133	–	–	–	–	–
$\mathbf{I_a}$	Me	$C_{10}H_{10}O_2$	162	35	0.76 [a]	86.9	74.02 (74.06)	6.21 (6.21)	–
$\mathbf{I_b}$	Et	$C_{11}H_{12}O_2$	176	269 [b]	0.71 [a]	73.2	74.88 (74.97)	6.80 (6.86)	–
$\mathbf{I_c}$	*n*-Pr	$C_{12}H_{14}O_2$	190	280 [b]	0.75 [a]	75.2	75.77 (75.76)	7.38 (7.41)	–
$\mathbf{I_d}$	I-Pr	$C_{12}H_{14}O_2$	190	261 [b]	0.87 [a]	69.4	75.69 (75.76)	7.35 (7.41)	–
$\mathbf{I_e}$	*n*-Bu	$C_{13}H_{16}O_2$	204	146 [b]	0.921 [a]	85.9	76.40 (76.44)	7.81 (7.89)	–
$\mathbf{I_f}$	I-Bu	$C_{14}H1_8O_2$	204	137 [b]	0.901 [a]	88.0	76.37 (76.44)	7.81 (7.89)	–
$\mathbf{I_g}$	CH_2–CH_2–$(CH_3)_2$	$C_9H_8O_2$	218	216 [b]	0.68 [a]	67.0	76.91 (77.03)	8.26 (8.30)	–
$\mathbf{I_h}$	CH_2–$(CH_2)_6$–CH_3	$C_{17}H_{24}O_2$	260	Above 300 [b]	0.74 [a]	74.0	78.38 (78.40)	9.28 (9.29)	–
$\mathbf{I_i}$	CH_2-Ph	$C_{16}H_{14}O_2$	238	75	0.91 [a]	75.0	80.59 (80.64)	5.92 (5.92)	–
$\mathbf{I_j}$	8-Hydroxy Quinolinyl	$C_{18}H_{13}O_2$	275	79	0.61 [a]	43.6	78.49 (78.52)	4.74 (4.76)	–
$\mathbf{I_k}$	Ph	$C_{15}H_{12}O_2$	224	73	0.91 [a]	29.0	80.37 (80.33)	5.36 (5.40)	–
$\mathbf{II_a}$	*m*-NO_2	$C_9H_7O_4$	193	194	0.62 [a]	73.1	55.91 (55.95)	3.62 (3.66)	7.23 (7.25)
$\mathbf{II_b}$	*p*-Ome	$C_{10}H_{10}O_3$	178	170	0.22 [c]	90.8	67.36 (67.40)	5.64 (5.66)	–
$\mathbf{II_c}$	Dibromo	$C_9H_8O_2Br_2$	308	111	0.53 [a]	47.0	35.26 (35.06)	2.58 (2.62)	–
$\mathbf{II_d}$	Dihydroxy	$C_9H_{10}O_4$	182	Above 300	0.45 [a]	96.4	59.19 (59.29)	5.52 (5.54)	–
$\mathbf{III_a}$	NH_2	C_9H_9NO	147	92	0.62 [d]	60.7	73.40 (73.44)	6.13 (6.17)	9.48 (9.52)
$\mathbf{III_b}$	Ph-NH	$C_{15}H_{13}NO$	223	112	0.24 [e]	30.0	80.59 (80.68)	5.90 (5.87)	6.31 (6.28)
$\mathbf{III_c}$	*o*-NO_2-Ph-NH	$C_{15}H_{12}N_2O_3$	268	60	0.89 [a]	44.8	67.11 (67.65)	4.49 (4.51)	10.32 (10.45)
$\mathbf{III_d}$	2,4-$(NO_2)_2$-Ph-NH	$C_{15}H_{11}N_3O_5$	313	110	0.23 [a]	30.6	57.46 (57.50)	3.46(3.54)	13.31 (13.42)
$\mathbf{III_e}$	*p*-Cl-Ph-NH	$C_{15}H_{12}NOCl$	257	170	0.11 [a]	61.5	70.20 (70.04)	4.63 (4.71)	5.36 (5.45)
$\mathbf{III_f}$	*o*-Cl-Ph-NH	$C_{15}H_{12}NOCl$	257	95	0.90 [a]	40.4	70.19 (70.04)	4.74(4.71)	5.33 (5.45)
$\mathbf{III_g}$	*m*-Cl-Ph-NH	$C_{15}H_{12}NOCl$	257	145	0.47 [c]	81.7	70.10 (70.04)	4.64 (4.71)	5.38 (5.45)
$\mathbf{III_h}$	*p*-OMe-Ph-NH	$C_{16}H_{15}NO_2$	253	85	0.68 [a]	69.6	75.73 (75.86)	5.87 (5.97)	5.49 (5.53)
$\mathbf{III_i}$	*o*-Me-Ph-NH	$C_{16}H_{15}NO$	237	170	0.21 [a]	40.5	80.82 (80.98)	6.28 (6.38)	5.81 (5.90)
$\mathbf{III_j}$	$N(CH_3)_2$	$C_{11}H_{13}NO$	175	120	0.33 [a]	46.9	75.48 (75.39)	7.46 (7.48)	7.68 (7.80)
$\mathbf{III_k}$	$N(C_2H_5)_2$	$C_{13}H_{17}NO$	193	72	0.11 [a]	20.7	76.78 (76.80)	8.24 (8.44)	6.81 (6.89)
$\mathbf{III_l}$	$N(C_2H_4OH)_2$	$C_{13}H_{17}NO_3$	225	49	0.10 [a]	46.2	66.52 (66.36)	7.21 (7.29)	5.91 (5.95)
$\mathbf{III_m}$	Morpholinyl	$C_{13}H_{15}O_2$	217	65	0.32 [a]	15.7	71.90 (71.86)	6.98 (6.96)	6.31 (6.45)
$\mathbf{III_n}$	Piperidinyl	$C_{14}H_{17}NO$	215	102	0.36 [a]	59.5	78.23 (78.09)	7.86 (7.97)	6.47 (6.51)
$\mathbf{III_o}$	I-Pr-NH	$C_{12}H_{15}NO$	189	65	0.60 [a]	39.7	76.41 (76.15)	7.87 (8.00)	7.28 (7.40)
$\mathbf{III_p}$	*n*-Bu-NH	$C_{13}H_{17}NO$	203	45	0.32 [a]	61.1	76.62 (76.80)	8.29 (8.44)	6.86 (6.89)
$\mathbf{III_q}$	NH–NH_2	$C_9H_{10}N_2O$	162	126	0.25 [a]	30.2	66.45 (66.64)	6.32 (6.18)	17.16 (17.28)

[b]Boiling point.

TLC MOBILE PHASE:
[a]Benzene.
[c]Benzene:ethylacetate(1:1).
[d]Ethyl acetate.
[e]Benzene:hexane(1:1).

Therefore to complete our study, we have performed a quantitative structure–activity relationship (QSAR) investigation in order to reach a better understanding of the different physicochemical properties. In principle, QSAR analysis is based on the assumption that the structurally similar compounds are similarly oriented and bind to the same biological site. The different QSAR techniques differ in the way of describing compounds and detecting the relationship between their structure and activity. The aim of multiple linear regression analysis is to find a statistically significant correlation between different physicochemical parameters of the compounds and their biological activity.

In the present study, multiple linear regression analysis method was applied to derive statistically significant quantitative models relating the structural features of cinnamic acid derivatives $\mathbf{I_{a–k}}$, $\mathbf{II_{a–d}}$ and $\mathbf{III_{a–q}}$ and their antimicrobial activity. Besides the explanatory ability of these models, they could be used to predict potency of compounds not yet tested and to suggest ideas for further synthesis of new molecules with enhanced activity.

2. Chemistry

The esters of $\mathbf{I_{a–i}}$ were synthesized by refluxing cinnamic acid with corresponding alcohols as reported in our previous work [7]. Esters $\mathbf{I_j}$ and $\mathbf{I_k}$ were prepared via the reaction of corresponding alcohols with acid chloride (Fig. 1). The substituents on cinnamic acid were chosen by introducing different groups, such as –NO_2, –OCH_3 that can be assumed to confer antimicrobial activity (Fig. 2). To study the effect of removal of double bond on antimicrobial activity, compound

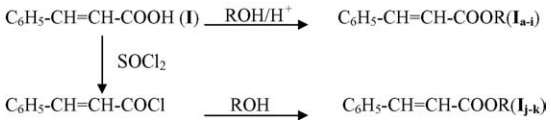

Fig. 1. General scheme for the synthesis of esters ($\mathbf{I_{a–k}}$).

Fig. 2. General scheme for the synthesis of ring substituted cinnamic acids ($\mathbf{II_{a–b}}$).

IIc and $\mathbf{II_d}$ were prepared [20,21] (Fig. 3). The amides

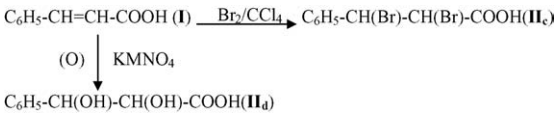

Fig. 3. General scheme for the side chain substituted cinnamic acids ($\mathbf{II_{c–d}}$).

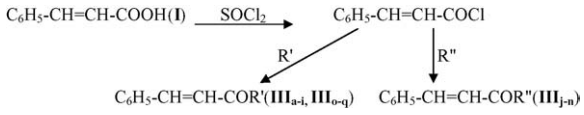

Fig. 4. General scheme for the synthesis of amides ($\mathbf{III_{a–q}}$).

($\mathbf{III_{a–q}}$) of cinnamic acid were synthesized by the reaction of cinnamoyl chloride with the corresponding primary or secondary amines [22] (Fig. 4).

The compounds $\mathbf{I_{a–k}}$, $\mathbf{II_{a–d}}$ and $\mathbf{III_{a–q}}$ were generally obtained in good yield and purified by recrystallization. Physicochemical data of these compounds and elemental analyses, for C, H, N, are shown in Table 1. Structural IR data is shown in Table 2. Structural ^{1}H-NMR data have only been given in experimental protocols for compounds $\mathbf{I_a}$, $\mathbf{II_b}$ and $\mathbf{III_a}$.

3. Biology

The in vitro antimicrobial activity of the synthesized compounds was assayed on bacterial and fungal strains and the minimum inhibitory concentration (MIC) was determined by serial dilution using salicylic acid as a reference compound.

4. Results

4.1. Antimicrobial activity

The log(1/MIC) values obtained for cinnamic acid derivatives $\mathbf{I_{a–k}}$, $\mathbf{II_{a–d}}$ and $\mathbf{III_{a–q}}$ as well as the parent compound, cinnamic acid (I) and the reference compound, salicylic acid are reported in Table 3. All the compounds reported in Table 3 showed a good antibacterial activity against Gram negative *Escherichia coli* than Gram positive *Staphylococcus aureus* and *Bacillus subtilis*. Compounds $\mathbf{I_f}$ and $\mathbf{II_c}$ proved to be the most effective, having a log(1/MIC) value of 2.73 against *E. coli*. In all other cases the assayed substances showed activity comparable to the standard salicylic acid.

Many of the tested cinnamic acid derivatives showed antifungal properties, displaying log(1/MIC) values of 2.55–3.10 against *Candida albicans* and *Aspergillus niger*. Compounds $\mathbf{I_f}$ and $\mathbf{II_c}$ showed activity equal to the reference compound (log 1/MIC = 3.10) against both the fungal strains tested. Compound $\mathbf{III_k}$ showed good activity against *A. niger* (log 1/MIC = 3.05). All the other compounds tested showed activity comparable to that of salicylic acid, the reference drug.

Compounds $\mathbf{I_f}$ and $\mathbf{II_c}$ were found to be the most effective antimicrobial substances displaying growth inhibition of all the tested microorganisms.

4.2. Quantitative structure–activity relationship

The antimicrobial activities of the compounds studied, presented as log 1/MIC values (Table 3), were used in QSAR studies. For the purpose of QSAR, the chemical structure of each compound was described by three groups of parameters: steric, electronic and hydrophobic which were selected due to their encouraging effect in describing antimicrobial activity in our previous study [7]. Kier and Hall [23] suggested the valence zero order molecular connectivity, a structural parameter that can be calculated by the following equation.

$$^0\chi^V = \sum^A (\delta i^v)^{-1/2}$$

where A is the number of hydrogen atoms and δi^v is the valence vertex degree of atom 'i' in hydrogen suppressed molecule. This connectivity index signifies the degree of branching, connectivity of atoms, and the unsaturation in the molecule, which accounts for the variation in the activity [24]. Topological indices are 2D descriptors [23], based on graph theory concepts which have been widely used in QSPR and most recently in QSAR studies, which help to differentiate the molecules according to their size, degree of branching, flexibility and overall shape. Global topological charge index [25,26], a recently developed topological indices can be calculated as

$$\text{Global topological charge index} = \sum_{(k-1)} \text{Ch.Jk}$$

where Ch.Jk is the topological charge index of order k. The hydrophobic parameter log P was computed by using CLOGP software. The other parameters like unsaturation index (U_i), hydrophilic factor (H_y), mean atomic Van der Waals volume (M_v), valance zero order molecular connectivity ($^0\chi^V$) and global topological charge index (JGT) were computed by using DRAGON software. Total energy (T_e) was computed from Hyperchem version 6. The values of the above parameters are presented in Table 4. The multiple linear regression analysis was performed by using QSAR-PC software. Table 5 presents the correlation matrix of the antimicrobial activity and Table 6 presents the comparison log(1/MIC) against different parameters. The equations obtained by multiple linear regression analysis are presented in Table 7.

Table 2
IR spectra of cinnamic acid derivatives

Compound code	IR (Nujol, ν cm^{-1})
I_a	1590 (C–C, aromatic), 1640–1650 (C=C, alkene disubstituted trans), 1730 (C=O, ester), 1280 (C–O, COOH), 730 (C–H, aromatic)
I_b	1590 (C–C, aromatic), 1640–1650 (C=C, alkene disubstituted trans), 1710 (C=O, ester), 1320 (C–O, COOH), 780 (C–H, aromatic)
I_c	1590 (C–C, aromatic), 1630–1650 (C=C, alkene disubstituted trans), 1690–1730 (C=O, ester), 1250–1340 (C–O, COOH), 780 (C–H, aromatic)
I_d	1590 (C–C, aromatic), 1620–1730 (C=O, ester), 770–780 (C–H, aromatic)
I	1590 (C–C, aromatic), 1630–1650 (C=C, alkene disubstituted trans), 1680–1730 (C=O, ester), 770–780 (C–H, aromatic)
I_f	1590 (C–C, aromatic), 1650 (C=C, alkene disubstituted trans), 1720 (C=O, ester), 1320 (C–O, COOH), 780 (C–H, aromatic)
I_g	1590 (C–C, aromatic), 1640–1650 (C=C, alkene disubstituted trans), 1690–1720 (C=O, ester), 780 (C–H, aromatic)
I_h	1590 (C–C, aromatic), 1640–1650 (C=C, alkene disubstituted trans), 1690–1720 (C=O, ester), 1310–1320 (C–O, COOH), 780 (C–H, aromatic)
I_j	1640 (C=C, aromatic), 1700 (C=C, alkene disubstituted trans), 1730 (C=O, ester), 1320 (C=N, aromatic), 730–740 (C–H, aromatic)
I_k	1610 (C–C, aromatic), 1650 (C=C, alkene disubstituted trans), 1740–1750 (C=O, ester), 1310–1320 (C–O, COOH), 730 (C–H, aromatic)
II_a	1640 (C=C, alkene disubstituted trans), 1700 (C=O, COOH), 1310 (C–N, C–NO_2 aromatic), 730 (C–H, aromatic)
II_b	1590–1610 (C–C, aromatic), 1620–1640 (C=C, alkene disubstituted trans), 1700 (C=O, COOH), 730 (C–H, aromatic)
II_c	1700 (C=O, COOH), 1600–1640 (C=C, alkene disubstituted trans), 730 (C–H, aromatic)
II_d	3350–3450 (C–OH, 3° alcohol), 1640 (C=O), 1550–1560 (C=C, aromatic), 1310 (OH, 3° alcohol), 730 (C–H, aromatic)
III_a	3400 (N–H, 1° amide), 1660–1770 (C=O, 1° amide), 1610 (C=C, alkene disubstituted trans), 1120 (C–N, aliphatic), 730–740 (C–H, aromatic)
III_b	1720 (C=O, 2° amide), 1700 (C=C, alkene disubstituted trans), 1630–1640 (C=C, aromatic), 1170–1180 (C–N, aliphatic), 720–730 (C–H, aromatic)
III_c	1590 (N–H, 2° amide), 1690 (C=O, 2° amide), 1640 (C=C, alkene disubstituted trans), 1320 (C–N, C–NO_2 aromatic), 1170–1180 (C–N, aliphatic), 730–740 (C–H, aromatic)
III_d	1730 (C=O, 2° amide), 1680–1710 (C=C, alkene disubstituted trans), 1640 (C=C, aromatic), 1320 (C–N, C–NO_2 aromatic), 1180 (C–N, aliphatic), 730–740 (C–H, aromatic)
III_e	1600–1610 (N–H, 2° amide), 1670–1680 (C=O, 2° amide), 1630–1640 (C=C, alkene disubstituted trans), 1160–1190 (C–N, aliphatic), 740 (C–H, aromatic)
III_f	1600 (N–H, 2° amide), 1670–1680 (C=O, 2° amide), 1650 (C=C, alkene disubstituted trans), 1170 (C–N, aliphatic), 740 (C–H, aromatic)
III_g	1580–1610 (N–H, 2° amide), 1670–1680 (C=O, 2° amide), 1170 (C–N, aliphatic), 740 (C–H, aromatic)
III_h	1590 (N–H, 2° amide), 1700 (C=O, 2° amide), 1640 (C=C, alkene disubstituted trans), 1160–1170 (C–N, aliphatic), 740 (C–H, aromatic)
III_i	1600 (N–H, 2° amide), 1670–1680 (C=O, 2° amide), 1630–1640 (C=C, alkene disubstituted trans), 1170 (C–N, aliphatic), 740 (C–H, aromatic)
III_J	1700 (C=O, 3° amide), 1640 (C=C, alkene disubstituted trans), 1170 (C–N, aliphatic), 740 (C–H, aromatic)
III_K	1670–1680 (C=O, 3° amide), 1630–1640 (C=C, alkene disubstituted trans), 1170 (C–N, aliphatic), 740 (C–H, aromatic)
III_l	1700 (C=O, 3° amide), 1640 (C=C, alkene disubstituted trans), 1170 (C–N, aliphatic), 740 (C–H, aromatic)
III_m	1660 (C=O, 3° amide), 1610 (C=C, alkene disubstituted trans), 1130–1140 (C–N, aliphatic), 740 (C–H, aromatic)
III_n	1160–1670 (C=O, 3° amide), 1640 (C=C, alkene disubstituted trans), 1170 (C–N, aliphatic), 740 (C–H, aromatic)
III_o	3350 (NH), 1560 (N–H, 2° amide), 1660–1670 (C=O, 2° amide), 1630 (C=C, alkene disubstituted trans), 1170 (C–N, aliphatic), 740 (C–H, aromatic)
III_p	1590 (NH, 2° amide), 1700 (C=O, 2° amide), 1640 (C=C, alkene disubstituted trans), 1160–1170 (C–N, aliphatic), 730–740 (C–H, aromatic)
III_q	1680–1710 (C=O, 2° amide), 1630–1650 (C=C, alkene disubstituted trans), 1160–1170 (C–N, aliphatic), 730–740 (C–H, aromatic)

5. Discussion

5.1. Antimicrobial activity

From the results of the microbiological studies, it is evident that both isobutyl cinnamate (**I_f**) and dibromo cinnamic acid (**II_c**) exhibited strong antibacterial activity against Gram positive and Gram negative bacteria and good antifungal properties. It is worthwhile observing that the removal of double bond in side chain of cinnamic acid was effected with –OH and –Br group. The results showed that addition of halogens to the side chain (**II_c**) caused remarkable increase in growth inhibitory effect of cinnamic acid whereas addition of hydroxy groups to the side chain (**II_d**) double bond did not remarkably enhance the antimicrobial activity.

5.2. QSAR studies

The QSAR studies were performed by considering log(BA) as dependent variable, where BA = 1/MIC[1]. A correlation matrix (Table 5) was constructed to check the interrelationship amongst the different parameters, which indicated that none of the parameters is closely related to any other parameter, $r < 0.7$ in all the cases.

Preliminary analyses were carried out in terms of correlation between log(BA) and each of the seven physicochemical parameters independently and the results are presented in Table 6. Amongst the bacterial species, all independent variables showed a poor correlation with a maximum r value being 0.42 (log(BA_s) with M_v). A similar trend was observed

[1] BA= (1/MIC)

Table 3
Antimicrobial activity of cinnamic acid derivatives, expressed as log(1/MIC) μg M ml^{-1}

Compound code	Log(1/MIC)				
	S. aureus	*B. subtilis*	*E. coli*	*C. albicans*	*A. niger*
I	2.34	2.34	2.39	2.69	2.69
I_a	2.29	2.25	2.39	2.69	2.64
I_b	2.29	2.34	2.34	2.69	2.64
I_c	2.25	2.34	2.34	2.65	2.73
I_d	2.43	2.39	2.43	2.73	2.73
I_e	2.34	2.34	2.34	2.64	2.78
I_f	2.69	2.73	2.73	3.05	3.10
I_g	2.34	2.34	2.39	2.73	2.73
I_h	2.34	2.39	2.29	2.73	2.64
I_i	2.29	2.34	2.34	2.73	2.69
I_j	2.34	2.34	2.39	2.59	2.64
I_k	2.39	2.29	2.39	2.64	2.69
II_a	2.29	2.34	2.29	2.73	2.73
II_b	2.34	2.34	2.39	2.69	2.69
II_c	2.73	2.73	2.73	3.10	3.10
II_d	2.13	2.43	2.39	2.55	2.59
III_a	2.29	2.29	2.29	2.64	2.64
III_b	2.34	2.25	2.25	2.55	2.59
III_c	2.43	2.39	2.34	2.59	2.55
III_d	2.39	2.39	2.39	2.55	2.69
III_e	2.25	2.25	2.25	2.64	2.69
III_f	2.25	2.25	2.25	2.64	2.69
III_g	2.34	2.34	2.39	2.64	2.73
III_h	2.39	2.34	2.34	2.69	2.69
III_i	2.43	2.48	2.43	2.55	2.59
III_j	2.43	2.43	2.43	2.73	2.69
III_k	2.29	2.34	2.34	2.78	3.05
III_l	2.25	2.25	2.25	2.55	2.64
III_m	2.34	2.39	2.34	2.64	2.64
III_n	2.43	2.39	2.39	2.69	2.73
III_o	2.29	2.39	2.39	2.69	2.64
III_p	2.39	2.34	2.34	2.73	2.73
III_q	2.34	2.39	2.34	2.59	2.59
S	2.39	2.39	2.39	3.10	3.1

Table 4
Physicochemical parameters of cinnamic acid derivatives

Compound code	Log *P*	U_i	H_y	M_v	$^0\chi^V$	JGT	T_e
I	2.09	3.17	–0.20	0.65	5.90	0.35	–0.45
I_a	2.47	3.17	–0.81	0.64	6.85	0.34	4.25
I_b	3.00	3.17	–0.82	0.62	7.56	0.32	4.89
I_c	2.52	3.17	–0.84	0.61	8.72	0.31	5.51
I_d	3.30	3.17	–0.84	0.691	8.43	0.41	5.43
I_e	4.05	3.17	–0.85	0.61	8.97	0.29	6.26
I_f	3.92	3.17	–0.85	0.61	9.41	0.40	6.06
I_g	4.45	3.17	–0.86	0.60	9.85	0.37	8.24
I_h	6.16	3.17	–0.88	0.59	11.8	0.23	8.70
I_i	4.23	3.90	–0.88	0.66	9.95	0.29	1.65
I_j	4.58	4.32	–0.84	0.69	11.27	0.38	15.32
I_k	4.31	3.90	–0.92	0.68	8.84	0.32	15.07
II_a	1.83	3.46	1.47	0.63	7.16	0.53	2.66
II_b	2.01	3.17	–0.19	0.63	7.23	0.46	6.50
II_c	2.88	3.00	–0.12	0.72	9.83	0.52	8.12
II_d	–0.39	3.00	1.48	0.61	6.79	0.52	9.28
III_a	1.43	3.17	–0.20	0.64	6.03	0.35	9.90
III_b	3.61	3.90	–0.37	0.67	9.34	0.31	17.97
III_c	3.45	4.09	–0.27	0.67	10.52	0.44	4.65
III_d	3.36	4.25	–0.19	0.67	11.71	0.55	4.70
III_e	4.79	3.90	–0.33	0.69	10.39	0.41	22.16
III_f	3.94	3.90	–0.33	0.69	10.39	0.401	19.18
III_g	4.79	3.90	–0.32	0.69	10.39	0.41	21.84
III_h	3.89	3.90	–0.35	0.65	10.67	0.39	12.47
III_i	3.81	3.90	–0.38	0.66	10.26	0.40	–9.77
III_j	1.73	3.17	–0.82	0.62	7.90	0.42	10.03
III_k	2.61	3.17	–0.85	0.60	9.31	0.41	20.36
III_l	0.78	3.17	0.42	0.60	9.62	0.38	3.62
III_m	1.57	3.17	–0.80	0.62	9.13	0.32	1.89
III_n	2.74	3.17	–0.86	0.61	9.43	0.32	8.15
III_o	2.59	3.17	–0.30	0.61	8.53	0.42	–2.84
III_p	3.34	3.17	–0.30	0.61	8.36	0.31	7.83
III_q	2.52	3.17	0.60	0.63	6.53	0.34	1.44

in case of fungal species with a maximum r value being 0.39 with U_i.

The multiple regression analysis showed a good correlation between log(BA) and U_i, JGT and log P. The regression equations (Table 7) obtained for the bacterial species showed the importance of constitutional parameter U_i, global topological charge index JGT followed by the lipophilic parameter log P in contribution to antibacterial activity (Eqs. (1)–(15)). The equations obtained by regression analysis were statistically significant in particular for *E. coli*.

For *E. coli*, the activity was strongly dependent on H_y and JGT and the relationships obtained were significant statistically (r = 0.552, P < 0.01, Eq. (12)). The addition of other parameters like U_i, log P, T_e, respectively, to the above parameters showed a gradual increase in r value which was significant statistically (P < 0.01, Eqs. (13)–(15)), which indicated the importance of these parameters in contribution to activity.

The regression equations obtained for fungal species were significant statistically (Eqs. (16)–(26)) in particular for *C. albicans* (Eqs. (16)–(20)). For *C. albicans*, the regression with single parameter indicated the importance of constitutional parameter U_i towards activity (P < 0.05, Eq. (16)). The inclusion of liphophilic parameter log P to U_i highly improved the statistical significance from P < 0.05 to P < 0.01 (Eq. (17)). This indicated the importance of log P in contribution to the activity. A similar trend was observed when JGT was added as a third parameter (P < 0.01, Eq. (18)).

The results of regression analysis showed the strongest dependence of activity were on constitutional parameter U_i, topological parameter JGT followed by lipophilicity parameter log P. The effect of steric parameter $^0\chi^V$ on activity was seen rarely as in case of *A. niger*, which may be considered as a chance correlation. Similarly the effect of electronic parameter T_e was seen rarely as it was added as fourth and fifth parameters with a marginal increase in r value. The importance of liphophilic parameter was well supported by the QSAR study of mono-, di-, tri-substituted cinnamic acids against *Listeria monocytogenes*, which indicated the importance of lipophilicity parameter and ionisation constant on activity [15].

Table 5
Correlation matrix for cinnamic acid derivatives against *S. aureus*

	Log(BA_s)	Log *P*	U_i	H_y	M_v	$^0\chi^V$	JGT	T_e
Log(BA_s)	1							
Log *P*	0.31	1						
U_i	–0.00	0.51	1					
H_y	–0.28	–0.56	–0.08	1				
M_v	0.42	0.53	0.40	–0.48	1			
$^0\chi^V$	0.27	0.69	0.61	–0.36	0.33	1		
JGT	0.17	–0.36	0.14	0.58	–0.20	–0.003	1	
T_e	–0.05	0.31	0.29	–0.15	0.08	0.29	0.004	1

Table 6
Comparison of log(BA) vs. parameters

	Log(BA_s)	Log(BA_B)	Log(BA_E)	Log(BA_C)	Log(BA_A)
Log *P*	0.31	0.02	0.05	0.17	0.15
U_i	–0.00	–0.24	–0.21	–0.39	–0.27
H_y	–0.28	0.001	–0.15	–0.22	–0.17
M_v	0.42	–0.07	0.03	0.19	0.18
$^0\chi^V$	0.27	–0.013	0.04	0.02	0.12
JGT	0.17	0.35	0.35	0.12	0.22
T_e	–0.05	–0.24	–0.15	0.06	0.14

In conclusion, some interesting compounds (**I_f, II_c**) endowed with high antimicrobial activity emerged from the present study. The cinnamic acid moiety is necessary for the studied activity. The constitutional parameter U_i, topological parameter JGT and the lipophilicity parameter log *P* can be considered as major factors of determining the differences in antimicrobial activity, but the other physicochemical indices are helpful for understanding the microbiological results, as shown by the QSAR analysis.

As the process of antimicrobial activity is not clearly defined in terms of target biomolecules, the QSAR results could not be addressed to a concrete drug–receptor interaction. However, they can reveal trends in the relationship between ligand structures and their activity for the set of antimicrobial agents selected. This will be useful for the future work in antimicrobial research and development.

6. Experimental

6.1. Chemistry

Melting points degree Celsius were determined with Elico melting point apparatus and are uncorrected. IR (Nujol) spectra were measured on Shimadzu IR 408 spectrophotometer. ^{1}H-NMR was recorded on FX-90Q FT-NMR spectrophotometer by using $CDCl_3$ as solvent and TMS as an internal standard (chemical shift in δ ppm). The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plates. Elemental analyses of (C, H, N) were obtained on a Vario-EL instrument.

6.1.1. General procedure for synthesis of compounds $I_{a–k}$

The appropriate alcohol (0.74 mol) was poured into a round bottom flask with cinnamic acid (0.08 mol) and sulphuric acid (2 ml). The solution was refluxed for 4 h. After completion of reaction, the reaction mixture was added in 200 ml of ice cold water and the oily layer was separated. The product was extracted with ether and evaporation of solvent resulted in product. The product was recrystallized from methanol. For the compounds **I_e–I_i**, the excess of alcohol from the product was removed by distillation at its boiling point.

Methyl cinnamate (**I_a**) IR:ν cm^{-1} = 1590 (C–C str., aromatic) 1640–1650 (C=C str., alkene disubstituted trans), 1730 (C=O ester), 1280 (C–O str.), 730 (C–H, aromatic). ^{1}H-NMR (300 MHz, $CDCl_3$):δ ppm = 3.8 (s, 3H, CH_3), 7.5 (m, 5H, Ar–H), 6.42–6.47, 7.67–7.73 (dd, H, CH=CH, J = 16.5 Hz).

6.1.2. General procedure for synthesis of p*-methoxy cinnamic acid ($II_{a–b}$)*

The appropriate aldehyde (0.015 mol) and malonic acid (0.035 mol) were dissolved in a mixture of 7.5 ml of pyridine and 1–2 drops of piperidine contained in a round bottom flask. The mixture was heated under reflux for 5 h. After the evolution of carbon dioxide ceased, the reaction mixture was poured into excess of water containing hydrochloric acid to remove excess of pyridine. The mixture was filtered and the product was recrystallized from alcohol.

p-Methoxy cinnamic acid (**II_b**) IR:ν cm^{-1} = 1590–1610 (C–C str., aromatic), 1620–1640 (C=C str., alkene disubstituted trans), 1700 (C=O str.), 730 (C–H, aromatic) ^{1}H-NMR (300 MHz, $CDCl_3$):δ ppm = 3.7 (s, 3H, CH_3), 7.5 (m, 4H, Ar–H), 6.82–6.88, 7.67–7.72 (dd, H, CH=CH, J = 0.051).

6.1.3. General procedure for synthesis of compounds $III_{a–q}$

The solution of corresponding amine (0.1 mol) in ether (50 ml) was added dropwise to a solution of cinnamoyl

Table 7
Equations derived by regression analysis for bacterial and fungal species

Equation no	Equation	Statistics			
		n	*R*	*s*	*F*
For S. aureus					
(1)	$\mathrm{Log}(BA_s) = 2.081(\pm0.108) + 0.445(\pm0.172)M_v$	33	0.422	0.103	6.73 [a]
(2)	$\log(BA_s) = 2.027(\pm0.120) - 0.106(\pm0.035)H_y + 0.760(\pm0.288)$ JGT	33	0.504	0.100	5.11 [a]
(3)	$\mathrm{Log}(BA_s) = 1.848(\pm0.150) - 0.074(\pm0.038)H_y + 0.341(\pm0.183)M_v + 0.070(\pm0.278)$ JGT	33	0.578	0.096	4.85 [b]
(4)	$\mathrm{Log}(BA_s) = 2.113(\pm0.163) + 0.044(\pm0.017)\log P\ 0.144(\pm0.050)U_I + 0.484(\pm0.173)M_v + 0.070(\pm0.278)$ JGT	33	0.669	0.089	5.68 [b]
(5)	$\log(BA_s) = 2.073(\pm0.164) + 0.037(\pm0.018)\log P - 0.132(\pm0.050)U_i - 0.047(\pm0.037)H_y + 0.408(\pm0.86)M_v + 0.927(\pm0.268)$ JGT	33	0.692	0.088	4.96 [b]
For B. subtilis					
(6)	$\mathrm{Log}(BA_B) = 2.179(\pm0.093) + 0.502(\pm0.238)$ JGT	33	0.354	0.101	4.43 [a]
(7)	$\mathrm{Log}(BA_B) = 2.419(\pm0.163) - 0.075(\pm0.043)U_i + 0.557(\pm0.233)$ JGT	33	0.455	0.098	3.92 [a]
(8)	$\log(BA_B) = 2.433(\pm0.146) + 0.046(\pm0.016)\log P - 0.161(\pm0.048)U_i + 0.913(\pm0.241)$ JGT	33	0.622	0.088	6.09 [b]
(9)	$\mathrm{Log}(BA_B) = 2.397(\pm0.141) + 0.052(\pm0.016)\log P - 0.150(\pm0.047)U_i + 0.945(\pm0.232)$ JGT $- 0.004(0.002)T_e$	33	0.675	0.084	5.85 [b]
(10)	$\mathrm{Log}(BA_B) = 2.340(\pm0.149) + 0.044(\pm0.017)\log P - 0.144(\pm0.047)U_i - 0.038(\pm0.034)H_y + 1.068(\pm0.225)$ JGT $- 0.004(\pm0.002)T_e$	33	0.693	0.083	4.99 [b]
For E. coli					
(11)	$\mathrm{Log}(BA_E) = 2.193(\pm0.091) + 0.447(\pm0.233)$ JGT	33	0.346	0.099	4.22 [a]
(12)	$\mathrm{Log}(BA_E) = 1.988(\pm0.107) - 0.089(\pm0.032)H_y + 0.898(\pm0.258)$JGT	33	0.552	0.089	6.56 [b]
(13)	$\mathrm{Log}(BA_E) = 2.259(\pm0.143) - 0.093(\pm0.037)U_i - 0.104(\pm0.030)U_y + 1.035(\pm0.243)$ JGT	33	0.656	0.082	7.29 [b]
(14)	$\mathrm{Log}(BA_E) = 2.304(\pm0.140) + 0.029(\pm0.016)\log P - 0.141(\pm0.044)U_i - 0.078(\pm0.032)H_y + 1.138(\pm0.240)$ JGT	33	0.701	0.079	6.76 [b]
(15)	$\mathrm{Log}(BA_E) = 2.277(\pm0.139) + 0.033(\pm0.016)\log P - 0.133(\pm0.044)U_i - 0.081(\pm0.031)H_y + 1.167(\pm0.237)$ JGT $- 0.003(\pm0.002)T_e$	33	0.720	0.077	5.98 [b]
For *C. albicans*					
(16)	$\mathrm{Log}(BA_C) = 3.085(\pm0.168) - 0.116(\pm0.049)U_i$	33	0.393	0.112	5.66 [a]
(17)	$\mathrm{Log}(BA_C) = 3.021(\pm0.157) + 0.045(\pm0.016)\log P - 0.190(\pm0.051)U_i$	33	0.580	0.101	7.62 [b]
(18)	$\mathrm{Log}(BA_C) = 2.889(\pm0.146) + 0.062(\pm0.015)\log P - 0.258(\pm0.047)U_i + 0.848(\pm0.234)$ JGT	33	0.737	0.085	11.50 [b]
(19)	$\mathrm{Log}(BA_C) = 2.889(\pm0.146) + 0.062(\pm0.015)\log P - 0.277(\pm0.045)U_i + 0.351(\pm0.160(M_v + 0.891(\pm0.221)$ JGT	33	0.781	0.080	10.96 [b]
(20)	$\mathrm{Log}(BA_C) = 2.855(\pm0.148) + 0.056(\pm0.016)\log P - 0.268(\pm0.045)U_i - 0.038(\pm0.034)H_y + 0.288(\pm0.168)M_v + 1.008(\pm0.242)$ JGT	33	0.793	0.079	9.13 [b]
For *A. niger*					
(21)	$\mathrm{Log}(BA_A) = 2.972(\pm0.172) - 0.079(\pm0.050)U_i$	33	0.273	0.115	2.50
(22)	$\mathrm{Log}(BA_A) = 2.944(\pm0.163) - 0.158(\pm0.059)U_i + 0.034(\pm0.015^0X^Y)$	33	0.452	0.18	3.86 [a]
(23)	$\mathrm{Log}(BA_A) = 3.063(\pm0.170) + 0.035(\pm0.017)\log P - 0.136(\pm0.055)U_i$	33	0.435	0.109	3.50 [a]
(24)	$\mathrm{Log}(BA_A) = 2.871(\pm0.153) + 0.065(\pm0.017)\log P - 0.209(\pm0.051)U_i + 0.910(\pm0.254)$ JGT	33	0.662	0.092	7.54 [b]
(25)	$\mathrm{Log}(BA_A) = 2.748(\pm0.162) + 0.055(\pm0.017)\log P - 0.227(\pm0.050)U_i + 0.322(\pm0.178)M_v + 0.951(\pm0.245)$ JGT	33	0.705	0.088	6.93 [b]
(26)	$\mathrm{Log}(BA_A) = 2.711(\pm0.165) + 0.048(\pm0.018)\log P - 0.216(\pm0.050)U_i - 0.044(\pm0.037)H_y + 0.251(\pm0.187)M_v + 1.082(\pm0.269)$ JGT	33	0.722	0.088	5.80 [b]

[a] $P < 0.05$.
[b] $P < 0.01$.

chloride (0.05 mol) in ether (50 ml). The solution was stirred for 30 min. The precipitated amide was separated and recrystallized from alcohol.

Cinnamide (**III$_a$**) IR:ν cm^{-1} = 3400 (N–H str., 1° amide), 1660–1670 (C=O str., 1° amide), 1610 (C=C str., alkene disubstituted trans), 1120 (C–N str., aliphatic), 730–740 (C–H bond, aromatic). ^{1}H-NMR (300 MHz, $CDCl_3$):δ ppm = 7.05 (s, 2H, –NH_2), 7.5 (m, 5H, Ar–H), 6.62–6.67, 7.54–7.61 (dd, H, CH=CH, J = 0.060).

6.2. Microbiology

The synthesized compounds were evaluated for their in vitro antimicrobial activity against Gram positive *S. aureus*, *B. subtilis*, Gram negative *E. coli* and also against fungi *C. albicans* and *A. niger*.

The MIC (μM ml^{-1}) was determined by using serial dilution technique [27,28] at different concentrations (2.5, 1.25... 0.078 μM ml^{-1}). Salicylic acid was used as reference. The compounds were dissolved in dimethyl sulfoxide. To the culture tubes containing 1 ml of medium, 1 ml of test solution was added under sterile conditions. To all the tubes including standard and controls, 0.1 ml of the fresh inoculum was added. The cells were incubated at 37 °C for 24 h (bacteria), at 30° for 48 h (*C. albicans*) and at 30 °C for 7 days (*A. niger*). The MIC was recorded in each case as the minimum concentration of compound, which inhibited the growth of

tested microorganism. From the MIC values observed, the intermediate concentrations between MIC values were prepared by serial dilution by using 0.1 μM ml^{-1} test solution and the accurate MIC values were determined. The antibacterial activity was tested by using double strength nutrient broth and antifungal activity was tested by using Sabouraud Liquid Medium. [2] All experiments were performed in triplicate.

6.3. Data analysis

6.3.1. QSAR

The QSAR parameter log *P* was generated from CLOGP program for windows software (Biobyte version 4.0, Claremont, CA). The other parameters like unsaturation index (U_i), hydrophilic factor (H_y), mean atomic Van der Waals volume (M_v), valence zero order molecular connectivity ($^0\chi^V$) and global topological charge index (JGT) were generated from DRAGON software. [3] Total energy (T_e) was computed by using Hyperchem version 6. [4] The MLR analysis was performed by QSAR-PC (medicinal chemistry regression program) software. [5]

Acknowledgements

The authors thank the Head, Analytical Department, University of Pune, for recording ^{1}H-NMR and the Head, Analytical Department, C-MET Electronics Ltd., Pune for C, H, N analysis. One of the authors (B.N.) is grateful to All India Council of Technical Education, New Delhi for the award of Junior Research Fellowship.

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